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Structure of killer cell immunoglobulin-like receptors and their recognition of the class I MHC molecules

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Immunological Reviews 2001
Vol. 181: 66–78

Immunological Reviews
ISSN 0105-2896

Summary: The recognition of class I MHC molecules by killer cell immunoglobulin-like receptors (KIR) constitutes an integral part of immune surveillance by the innate immune system. To understand the molecular basis of this recognition, the structures of several members of this superfamily have been determined. Despite their functional diversity, members of this superfamily share many conserved structural features. A central question is how these receptors recognize their ligands. The recent determination of the crystal structure of KIR2DL2 in complex with HLA-Cw3 has revealed the molecular mechanisms underpinning this interaction, which ultimately modulates the cytolytic activity of natural killer cells. While the recognition of MHC molecules by KIR is characterized by a number of unique features, some unexpected similarities with T-cell receptor recognition of MHC molecules are also observed. The detailed interactions between KIR2DL2 and HLA-Cw3 and their functional implications will be reviewed here.

Introduction

The cytolytic activity of natural killer (NK) cells is regulated by both activating and inhibitory receptors on the cell surface. Although the ligands for many activating receptors remain unknown, the ligands for a number of inhibitory receptors have been identified as class I MHC molecules mostly presenting self peptides (1, 2). To date, three distinct families of class I MHC-recognizing NK-cell receptors, Ly49, CD94/NKG2 and killer cell immunoglobulin-like receptors (KIR), have been identified. Structurally, Ly49 and CD94/NKG2 are members of C-type lectin-like receptor (CTLR) superfamily whereas KIR are members of immunoglobulin-like superfamily. The Ly49 receptor family, found only in mice, appears to be functionally equivalent to KIR, which in contrast to Ly49 have been observed in primates but not rodents (3). Both Ly49 and KIR are known to recognize classical class I MHC molecules. In contrast the heterodimeric CD94/NKG2 receptors are conserved throughout the species and recognize the non-classical class I MHC molecules, HLA-E in humans and Qa-1 in mice (4–7). Another homodimeric CTLR, NKG2D,

has recently been shown to bind class I-like molecules such as MHC class I chain-related A/B (MICA/B) and UL16 binding proteins 1–3 in humans together with retinoic acid-inducible early gene-1 (RAE-1) protein and H60 in mice (8–11).

The KIR superfamily

KIR are type I transmembrane glycoproteins with two or three extracellular C2-type (also referred to as K-type) immunoglobulin (Ig) domains (12–14). Receptors containing two or three Ig domains exist as either inhibitory or non-inhibitory forms (15–18). The inhibitory forms of these receptors contain immunoreceptor tyrosine-based inhibitory motifs (ITIM) in their cytoplasmic tail, whereas the non-inhibitory forms have a shorter cytoplasmic tail and contain a positively charged residue in the transmembrane region (1, 2). Recently, a unified nomenclature was proposed to name the members of KIR according to the number of Ig domains present in each gene and whether the C-terminal cytosolic tails contain ITIM (19). For example, KIR3DL1 represents an inhibitory receptor with three Ig domains and a 'long' intracellular tail possessing ITIM sequences whereas KIR3DS1 represents a non-inhibitory form with a 'short' cytoplasmic tail containing no ITIM segments. The consecutive domains are named D1 and D2 in KIR2D and D0, D1 and D2 in KIR3D receptors, respectively. Members of the KIR family share greater than 90% sequence identity. With the exception of KIR2DL4 and KIR2DL5, the second and third domains of the three-domain KIR are best aligned against the first and second domains of the two-domain KIR. KIR2DL4 possesses a D0 and D2 domain. Human KIR are encoded by approximately 12 genes located in the leukocyte receptor complex (LRC) region on chromosome 19q (20, 21). A similar number of *KIR* genes have been identified in other primate species (22). Since the discovery of *KIR* genes, several other cell surface receptors that share homology with KIR have also been identified. These include Ig-like transcripts (ILT) or leukocyte Ig-like receptors (LIR) (23–25), leukocyte-associated Ig-like receptors (LAIR) (26), paired Ig-like receptors (PIR) and gp49 (27–29). Among them, ILT contain two or four Ig-like domains; LAIR-1 and -2 contain a single Ig-like domain; PIR-A and PIR-B each contain six Ig-like domains. They display 35–50% sequence identity with KIR and clearly share a common fold with KIR. Together with KIR, they define the so-called KIR superfamily. A more distantly related set of proteins are the Ig-like Fc receptors (FcαR, FcγR-I, -IIa, -IIb, -III and FcεRI), which display less than 20% sequence identity with KIR but nonetheless share a structural fold similar to KIR. Consequently they are sometimes also regarded as part of the KIR superfamily.

The structure of KIR

To date, the crystal structures of the extracellular domains of three members of the KIR family, KIR2DL1, KIR2DL2 and KIR2DL3, have been published (30–32). Overall, the KIR fold is very similar to the C2-type Ig-like fold observed in the hematopoietic receptors, with the difference being primarily in the pairing of two β-strands (*Fig. 1*). In hematopoietic receptors, β-strand A pairs with the strand B, whereas in KIR structures the first strand splits into two strands, A and A′, which hydrogen bond with the B and G strands, respectively, resulting in "strand switching". This strand switching is likely attributable to the presence of a *cis*-proline residue in the first strand that is conserved in all members of KIR. Secondly, the KIR fold contains an additional short strand D that is absent in other C2-type Ig-like folds. In addition to the slight differences in strand arrangement, the KIR structures possess unique tertiary packing. In particular, the hinge angle between the N-terminal D1 and C-terminal D2 domains is smaller than those observed in other two Ig-like domain structures, such as human growth hormone receptor, the V and C domains of T-cell antigen receptors (TCR), and the V and C_{H1} domains of antibodies. The hinge angle of KIR varies from 66° in KIR2DL1 to 81° in KIR2DL2 and KIR2DL3. This small interdomain hinge angle is stabilized by a highly conserved interdomain hydrophobic core (hinge core) that consists of Leu17, Met69, Val100, Ile101, Thr102, His138, Phe178, Ser180, Pro185, Tyr186 and Trp188. An interdomain salt bridge between Asp98 and Arg149, conserved in all KIR family sequences, also helps to restrict the hinge angle. The D1 and D2 domains themselves share 40% sequence identity and appear to be the result of gene duplication. The superposition between the Cα atoms of D1 and D2 domains results in a root-mean-square (rms) deviation of approximately 1.2 Å. Aside from the differences in the hinge angle, the structures of KIR2DL1, KIR2DL2 and KIR2DL3 are nearly identical, with rms deviations less than 1 Å between the Cα atoms of their respective domains.

Other members of the KIR superfamily

Recently, Chapman et al. have determined the structure of the first two domains of LIR-1 (ILT-2) (33), an inhibitory receptor expressed on monocytes, B cells, dendritic cells and subsets of NK and T cells (23, 24). Overall, the sequence homo-

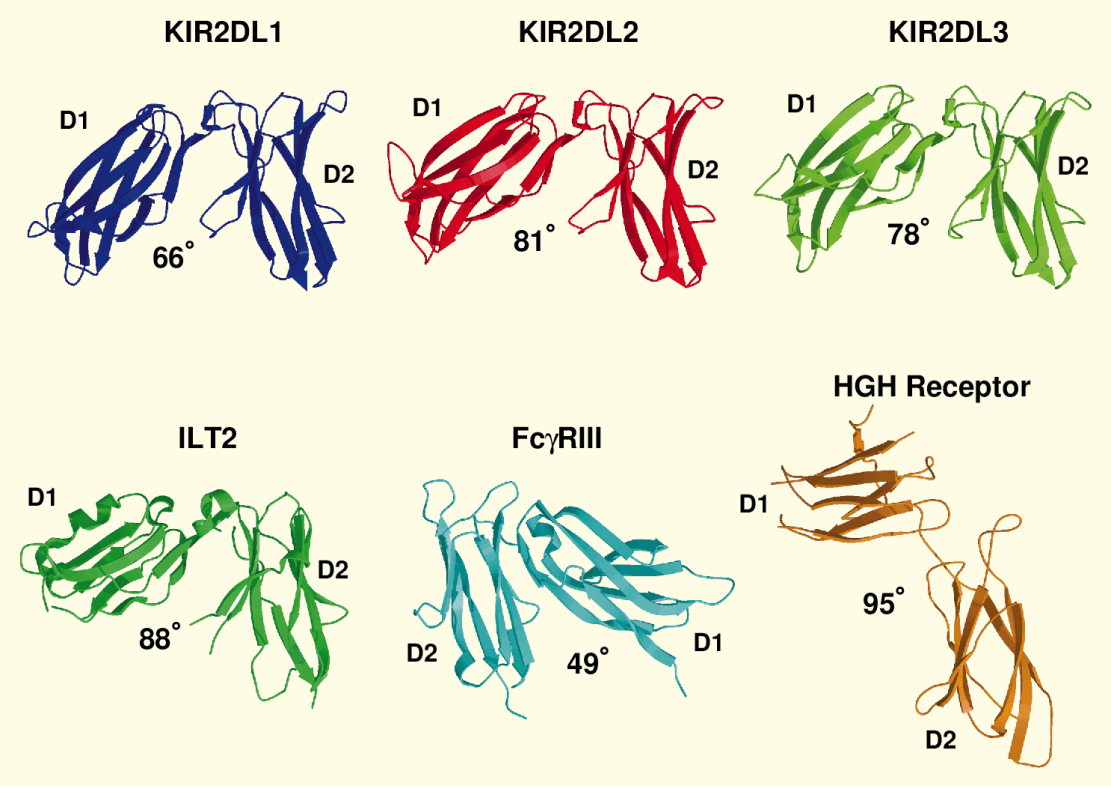

***Fig. 1.* Comparison of KIR receptors with other representative two-domain Ig-like receptors.** The D2 domains of each structure are all in exactly the same orientation. The hinge angles between the D1 and D2 domain are indicated for each receptor. Protein Data Bank (PDB) codes: KIR2DL1, 1NKR; KIR2DL2, 1EFX; KIR2DL3, 1B6U; ILT2, 1GOX; FcγRIII, 1FNL; human growth hormone (HGH) receptor, 3HHR. All molecular ribbon representations were created with MOLSCRIPT (83) and RASTER3D (84).

logies between *KIR* and *ILT* genes are about 40%, and domains 1 and 2 of LIR-1 possess the KIR-type Ig fold, including strand switching in the first β-strands. However, distinct structural differences are observed between LIR-1 and KIR (33). In particular, LIR-1 has two unique short 3_{10} helices in each domain. One replaces the C′ strand in the D1 domain and the C-terminal end of C′ strand in the D2 domain found in KIR, and the other is situated between the E and F strands in the D1 domain and between the F and G strands of the D2 domain (*Fig.* 1). A short left-handed type II polyproline-like helix is also found in the F–G loop of the D1 and D2 domains. Like KIR, the interdomain region of LIR-1 is occupied by primarily hydrophobic residues forming a hinge core that stabilizes the D1–D2 interdomain conformation. Interestingly, a conserved interdomain salt bridge in KIR between Asp98 and Arg149 is absent in the LIR-1 structure, possibly contributing the slightly larger hinge angle of LIR-1 (88°).

Somewhat distantly related to KIR and ILT are the Ig-like Fc receptors. Despite sharing less than 20% sequence identity with KIR, the fold of Fc receptors, as seen in the structures of FcγRIIa, FcγRIIb, FcγRIII and FcεRI, is observed to resemble that of KIR (34–37). For example, the characteristic strand switch found in the first β-strand of KIR domains is also present in the Fc receptor structures. Furthermore, the interdomain region of Fc receptors also contains a hydrophobic hinge core and an interdomain salt bridge, although the residues that contribute to the hinge core and the salt bridge are different between FcR and KIR. The individual domains of FcR can be superimposed with those of KIR, resulting in rms differences between Cα positions of approximately 2.0 Å. On the other hand, the FcR structures have hinge angles varying between 48° and 55°, significantly smaller than those found in KIR or LIR-1. In addition, the D1 domain of FcRs is located on the opposite side of the D2 domain relative to what is observed in KIR molecules (*Fig.* 1).

The class I MHC ligands of KIR

Class I MHC molecules were first implicated as potential ligands of NK-cell receptors when an inverse relationship was

identified between the susceptibility of target cells to NK-cell-mediated lysis and the level of expression of class I molecules on target cells (38, 39). The observation that transfection of class I genes into a class I-deficient target cell was sufficient to protect these cells from NK-cell-mediated lysis further strengthened these findings (40). Evidence for the involvement of multiple receptors that recognized distinct HLA class I molecules came from studies examining the specificity of cloned NK cells against different allogeneic target cells. This led to the identification of two 58 kDa proteins originally termed p58. The expression of these p58 molecules correlated with the allospecificity of cloned NK cells for target cells. In particular, NK clones that expressed the p58 molecule recognized by the EB6 mAbs (15, 41) were unable to lyse target cells expressing the HLA-Cw2, 4, 6 or 15 allotypes. Similarly NK cells expressing the other p58 molecule recognized by the GL183 mAb were unable to lyse target cells expressing HLA-Cw1, 3, 7 or 8 molecules (42, 43). Sequence comparison among these allotypic HLA molecules suggested that a dimorphism at residues 77 and 80 in class I MHC heavy chain accounts for the observed NK-cell lytic specificities (44, 45). A similar region in HLA-B molecules that contain the Bw4 serological epitope was also shown to confer protection from NK cells bearing a larger 70 kDa receptor (p70) (17, 46). However other regions of class I are almost certainly required for a productive interaction with p70, since the HLA-A2403 and -A2501 alleles that possess the Bw4 epitope are unable to protect the target cell from lysis by p70 expressing NK cells (46). The cloning of these p58 and p70 molecules led to the identification of a multigene family now known as KIR. The p58 receptors have been subsequently renamed KIR2DL1 (specific for HLA-Cw2, 4, 6 and 15) and KIR2DL2 and KIR2DL3 (specific for HLA-Cwl, 3, 7 and 8). The p70 receptor that interacts with HLA-B alleles of the Bw4 allotype was renamed KIR3DL1 (12, 13, 47, 48). The interactions between KIR2DL1 and HLA-Cw4 allotypes and between KIR3D receptors and HLA-Bw4 allotype have been observed directly using soluble forms of receptors (47, 49–53). Although there is no direct binding evidence, some KIR3D receptors have also been suggested to recognize certain HLA-A alleles (54, 55). Recently, the ligand for KIR2DL4 has been identified as the non-classical class I molecule HLA-G (56).

Structure of the KIR2DL2/HLA-Cw3 complex

The crystal structure of KIR2DL2 in complex with HLA-Cw3 and a nonamer self peptide GAVDPLLAL (GAV) derived from the importin-α1 subunit has been determined to 3.0 Å resolution (57). KIR2DL2 binds to HLA-Cw3 in the crystal in 1:1 stoichiometry, consistent with analytical equilibrium ultracentrifugation experiments in which the sedimentation curves were best fitted by a 1:1 binding model (P. Sun, P. Schuck, unpublished data). The orientation of KIR with respect to HLA is very similar to that of TCR, with the D1 and D2 domains of KIR assuming the respective positions of the Vα and Vβ domains of TCR (*Fig.* 2) (58). The footprint of KIR on the class I HLA overlaps partially with footprints of TCR on class I MHC molecules. However, KIR contacts primarily the P7 and P8 positions of the bound peptide and associated HLA residues, whereas TCR interaction with class I MHCs is centered on the P4, P5 and P6 positions of the peptide (59, 60). The KIR/HLA complex buries nearly 1,600 $Å^2$ of surface area, similar to that buried in a TCR/MHC complex. There are no significant conformational changes in either KIR2DL2 or HLA-Cw3 when the complexed structure is compared to its uncomplexed components. This suggests a rigid body association, which is consistent with both the rapid binding kinetics and the favorable entropic contributions to binding observed in KIR/MHC interactions (53, 57). In contrast, TCR/MHC interaction entails conformational changes in the TCR binding loops and is characterized by slower binding kinetics and unfavorable binding entropy (61, 62).

KIR interacts with HLA-Cw3 via six surface loops that are topologically identical to the six hormone binding loops on the human growth hormone receptor. Three of these loops (the A′B, CC′ and EF loops) are from the D1 domain and interact with the α1 helix of HLA-Cw3 and the GAV peptide. The hinge loop and the BC, FG loops of the D2 domain contact the α2 helix of HLA-Cw3. Unlike TCR/MHC interfaces that consist largely of hydrogen bonds, hydrophobic interactions and van der Waals interactions, the KIR/HLA interface is characterized by strong charge complementarity. In all, there are six acidic residues in KIR that interact with six basic residues in HLA-Cw3, resulting in the formation of four salt bridges between E21, E106, D135, D183 of KIR and R69, R151, R145, K146 of HLA-Cw3. The dominance of charge–charge interactions in the interface resembles the interface between adhesion receptors, such as CD2/CD58 (63). To evaluate the contribution of these salt bridges to the KIR/HLA recognition, single receptor mutants, E106A, D135H and D183A, were created to remove three of the four salt bridges individually, and their effects on HLA binding were measured by surface plasmon resonance (SPR). These mutations resulted in drastic reductions in the class I binding affinity of KIR (*Table 1*), suggesting a high binding energy threshold for

Fig. 2. Comparison of KIR/MHC binding (left) to TCR/MHC binding (right). The top panel shows the front view, the middle panel shows the side view and the bottom panel illustrates the top view of each complex. For reasons of clarity the bottom view shows only the α1α2 domains of the MHCs and only the Vα and Vβ domains of the TCR. The MHC molecules are green and yellow with red peptides, KIR is blue and the TCR has a blue α-chain and a violet β-chain. The TCR/MHC complex is represented by the human B7 TCR in complex with HLA-A2 and the TAX peptide (PDB code 1BD2). The PDB code for the KIR2DL2/HLA-Cw3 complex is 1EFX.

***Table* 1. Peptide and receptor mutation effects in KIR2DL2/HLA-Cw3 association**

Peptide	K_D (μM)	W6/32 binding (%)
Effects of peptide variation in KIR/HLA binding		
G A V D P L L A L (GAV)	9.5	100
– – – – – – – S – (GAV_S)	42.3	147
– – – – – – – V – (GAV_V)	525	130
– – – – – – – K – (GAV_Y)	>600	130
– – – – – – – K – (GAV_K)	>600	139
A A A D A A A A L (AAA)	48.5	149
T A MD V V Y A L (TAM)	38	138
Q A I S P R T L (QAI)	74	38
HLA-E	>600	84

Effects of amino acid substitutions in KIR/HLA association	
KIR mutant	K_D (μM)
Wild type	28
R33A	30
K44M	>400
Y105A	>400
E106A	185
D135H	>400
D183A	>400

KIR/HLA recognition. This low tolerance to interface mutations may be a reflection of the low affinity nature of this receptor/ligand interaction and its fast binding kinetics. In addition to charge complementarity, the KIR/HLA interface also contains hydrophobic interaction and eight hydrogen bonds. The largest hydrophobic cluster includes the aliphatic portions of K44, F45, M70 and Q71 of KIR2DL2 together with the aliphatic portions of V76, R69, R75 and R79 of HLA-Cw3. Substitution of Phe 45 with Tyr in KIR, as occurs naturally in KIR2DS2, reduces the HLA affinity of KIR significantly (42). Structurally, the close packing between Phe 45 of KIR and the Cβ atom of Arg 79 of HLA-Cw3 leaves little space and no potential hydrogen bonding partner to accommodate the hydroxyl group of Tyr. It is possible that this type of subtle difference in the interface packing accounts for the observed lower affinity in the non-inhibitory versus the inhibitory KIR.

Class I allotypic recognition of KIR

Early studies revealed that the expression of a number of different class I molecules was sufficient to protect target cells from NK-cell lysis. These data suggested that either there were distinct receptors for each class I allele or that this recognition was not specific for individual MHC/peptide complexes but rather for groups of related class I molecules complexed with a variety of peptides (45). It is now clear that multiple alleles

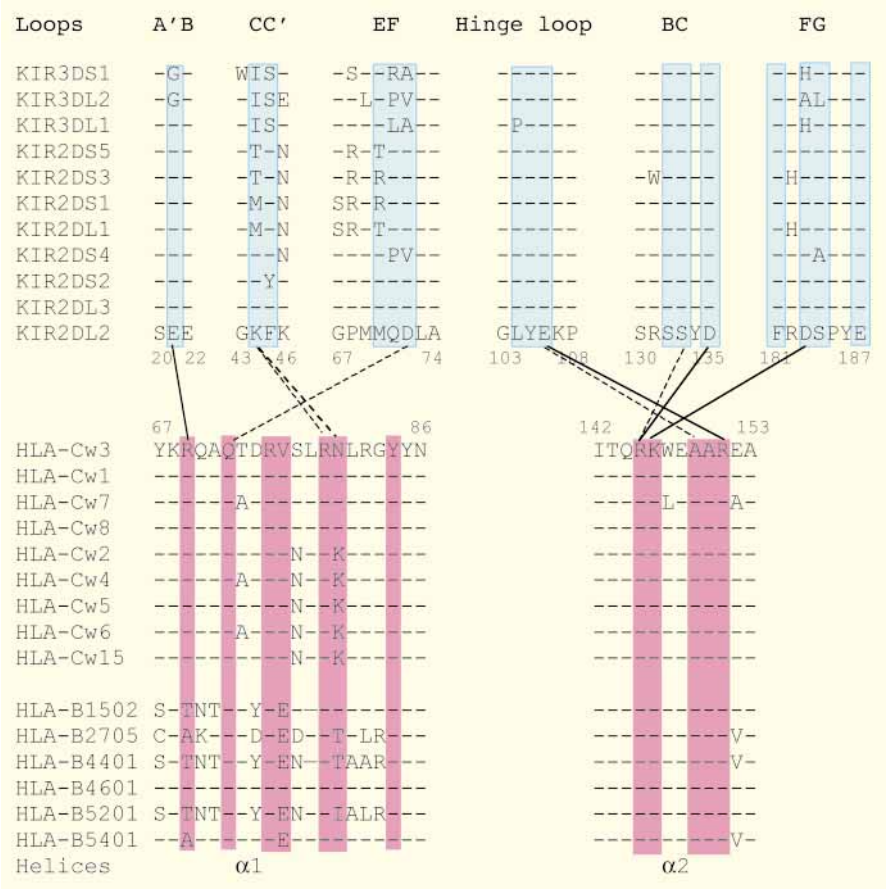

***Fig. 3.* Sequence alignments of KIR and HLA binding regions.** KIR alignments include the KIR2D and KIR3D proteins aligned with the six binding loops of KIR2DL2. Contact residues are shaded in blue. HLA alignments include regions of the α1 and α2 α-helices of HLA-C and HLA-Bw4 allotypes that align with contact regions on HLA-Cw3. Contact residues are shaded pink. The four interface salt bridges are represented with solid lines and five key interface hydrogen bonds are shown by dotted lines connecting the residues.

of MHC molecules can be recognized by the same receptor. In the case of KIR recognition of HLA-C, the specificity is largely determined by amino acid 80 of the heavy chain (64, 65). Similarly, the p70 receptor (KIR3DL1) recognizes multiple HLA-B alleles with the Bw4 serological epitope, which spans residues 77–83 of the class I heavy chain. Substitutions within this region significantly impact its recognition by KIR3DL1 (46, 64). Furthermore, the same class I MHC molecule can be recognized by multiple KIR. For example, KIR2DL1 and KIR2DS1 both interact with HLA-Cw2, 4, 5, 6 and 15, and KIR2DL2, KIR2DL3 both recognize HLA-Cwl, 3, 7 and 8 allotypes. The basis of this allotypic recognition of KIR was not understood until the structure of the KIR/HLA complex was solved. The KIR/HLA binding interface contains 12 residues from HLACw3. Eleven of them are invariant across all HLA-C alleles despite their location within the polymorphic region of the class I heavy chain. Amino acid 80 is the only variable residue contributing to the receptor interface (*Fig.* 3). Moreover, seven of the class I interface residues are also conserved in HLA-B allotypes. Interestingly, HLA-B46, which contains an HLA-Cw1 sequence between residues 66 and 76, has a putative KIR binding site identical to that of HLA-Cw1 and is recognized by NK cells that express HLA-C-specific receptors (66). In contrast, eight out 16 HLA-A2 residues in contact with the A6 TCR are variable among the HLA-A alleles.

The use of highly conserved residues within an otherwise polymorphic region of HLA enables individual KIR to recognize multiple class I HLA molecules while discriminating among various allotypes based on the identity of amino acid position 80. The recognition of conserved residues of HLA by KIR has important ramifications and may reflect the functional differences between the innate and adaptive immune systems. T cells of the adaptive immune system rely on TCR gene rearrangement to produce a population of clonally distinct effector cells. This is followed by antigen-driven selection and expansion of individual T cells that express a receptor with exquisite specificity for a particular MHC molecule complexed to a specific peptide. The diverse TCR repertoire that results from gene rearrangement combined with the polymorphism in MHC molecules enables T-cell-mediated cellular immunity to counter a vast array of pathogens. NK

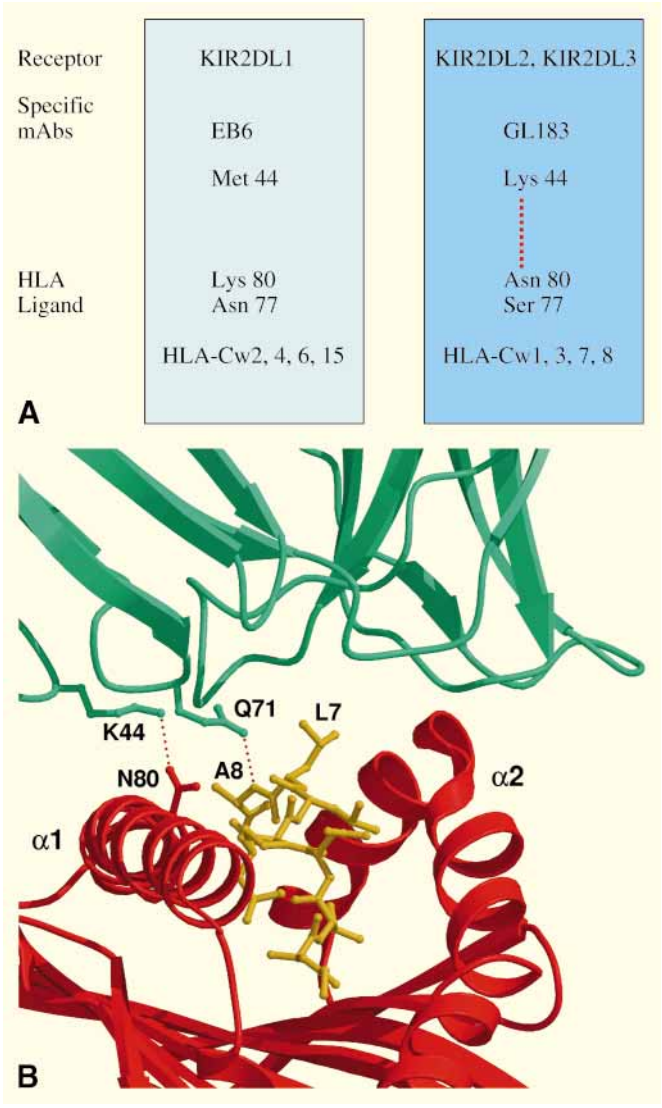

***Fig. 4.* A. Schematic diagram illustrating the allotype specificity of KIR2D receptors.** The shaded areas encompass the two known receptor/ligand subgroups in KIR2D allorecognition. Amino acids specific for this interaction are also listed. The red dotted line represents a hydrogen bond. **B. Critical hydrogen bonds between KIR2DL2 (green) and HLA-Cw3/GAV (red and yellow).** Dotted lines depict the hydrogen bond between Lys44 and Asn80 which confers allotypic specificity and the one hydrogen bond between the receptor and the peptide.

cells, as part of the innate immune system, help provide a rapid first line of defense against pathogens. In this context there is limited time to select and expand specific clonal populations. Thus, NK-cell receptors, in contrast to TCR, are germline coded and have limited ability to adapt to changing MHC and peptide combinations. Furthermore, there are significantly more MHC alleles than there are KIR receptors. Therefore, effective surveillance of MHCs by KIR requires individual KIR to recognize more than one MHC allele. KIR accomplishes this by recognizing the conserved residues within the polymorphic regions of MHCs. The use of the conserved residues for KIR recognition and polymorphic residues for TCR recognition enables a given class I MHC to effectively fulfill the recognition requirements for both the innate and adaptive immune systems. There are about a dozen *KIR* genes encoded by the human LRC (67). Several display limited polymorphism (20, 22, 68). The putative class I MHC contact residues of KIR, as defined in the structure of KIR2DL2/HLA-Cw3 complex, are well conserved and do not include the polymorphic KIR residues (data not shown). Among the various *KIR* gene products, the class I interface residues of KIR2DL2 are more conserved in the D2 domain than the D1 domain. This mirrors the conservation in their class I counterparts. Namely, the contact residues on the α2 helix of the class I MHC heavy chain, which interact with the residues on the D2 domain of KIR, are more conserved than those on the α1 helix (*Fig.* 3). The KIR2DL2 interface residues are completely conserved in KIR2DL3, which is consistent with both receptors recognizing the same HLA class I alleles. Similarly, except for a Phe 45 to Tyr replacement, all other interface residues of KIR2DL2 are conserved in KIR2DS2 as well. Fourteen of the 16 MHC contact residues of KIR2DL2 are conserved in 2DL1, 2DSl, 2DS3 and 2DS5, suggesting their class I binding mode is similar to that of 2DL2/Cw3.

In addition, KIR displays precise specificity for particular MHC allotypes. Remarkably, this is achieved through variation at a single KIR residue at position 44. This variation in KIR is sufficient to discriminate between polymorphisms found at position 80 of the HLA-C heavy chain. In this situation, KIR2DL1, containing a Met at position 44, recognizes HLA-C allotypes with Lys at position 80. KIR2DL2, on the other hand, has a Lys at position 44 and does not recognize HLA-C molecules with a Lys at position 80. Instead, it recognizes HLA-C allotypes with Asn at position 80 (*Fig.* 4*A*). In the crystal structure of KIR/HLA complex, Lys44 of KIR2DL2 makes a hydrogen bond with Asn80 of HLA-Cw3 (*Fig.* 4*B*). Replacing Asn80 with Lys, as in the sequence of HLA-Cw2, 4, 5 and 6, would generate unfavorable electrostatic interaction with Lys44 of KIR2DL2. Similarly, replacing Lys44 of KIR2DL2 with Met would result in the loss of the (KIR) Lys44-Asn80 (HLA) hydrogen bond and destabilize the KIR/HLA interface. Consequently, it seems that the HLA-C allotype specificity of KIR2D receptors is controlled by one hydrogen bond between the residue 44 of KIR and residue 80 of HLA-C heavy chain.

KIR3DL1/HLA-Bw4 interaction has also been explored. Sequence alignments show that ten of 16 KIR2DL2 interface residues and nine of 12 HLA-Cw3 interface residues are conserved in KIR3DL1 and the HLA-Bw4 allotype respectively. This provides for the potential of two of the four interface salt bridges and four of the eight interface hydrogen bonds observed in the KIR2DL2/HLA-Cw3 structure to be conserved in KIR3DLI/HLA-Bw4 recognition. This degree of conservation at the KIR/HLA interface suggests that the mode of binding of KIR3DL1 to HLA-Bw4 probably resembles that of KIR2DL2 to HLA-Cw3, and that the relative orientation and positioning of KIR with respect to HLA is likely more conserved than TCR/HLA recognition.

The peptide preference for KIR/HLA binding

In addition to the class I MHC residues, the associated peptides are also crucial for KIR recognition (12). Peptides are involved not only in the generation of stable, properly folded MHC molecules at the cell surface, but also in the direct recognition of class I MHC by NK cells. Cells deficient in transporter associated with antigen processing (TAP) cultured at 26 °C in the absence of class I binding peptides express "empty" class I MHC molecules on their cell surface. These class I molecules are unable to protect the target cells from lysis, indicating a requirement for peptide beyond merely stabilizing the class I molecule. Moreover, it is also evident that peptides vary considerably in their ability to enable a functional interaction between KIR and class I molecules. The KIR preference for peptides of certain sequence was first demonstrated in studies examining NK-cell recognition of HLA-B27-expressing target cells. These studies found that certain residues at P7 and P8 positions of the peptide failed to confer HLA-B27-dependent protection from NK-cell-mediated lysis. In particular, the charged residues Lys, Arg, Asp and Glu at positions P7 and P8 rendered class I MHC more susceptible to lysis (69, 70). Peptide preferences were also observed in KIR2D recognition of HLA-C molecules (50, 71). The crystal structure of the KIR2DL2/HLA-Cw3 complex contains a nonamer peptide (GAV) derived from the human importin-α1 subunit (71). The bound peptide displays the characteristic class I MHC peptide binding motif, in which the termini are secured by a conserved set of hydrogen bonds, the P2 and P9 residues are anchored in binding pockets, and middle P4–P7 positions form an arch above the floor of the HLA-Cw3 cleft. Interestingly, one conserved hydrogen bond, observed in the structures of HLACw4 and other class I MHC between Lys146 of HLA and the P9 carboxylate (72), is missing in the KIR/HLA complex structure. Instead, Lys146 of HLA-Cw3 forms a salt bridge with Asp183 of KIR2DL2.

KIR2DL2 makes direct contacts to the GAV peptide at both the P7 and P8 positions. The side chain of the P7 leucine points toward the receptor and forms hydrophobic interactions with Leu104 and Tyr105 of the KIR. This hydrophobic packing, however, is sufficiently loose to accommodate other amino acids, such as Tyr, observed in another protective peptide. At the P8 position, Gln 71 of KIR forms a hydrogen bond with the backbone amide nitrogen of the peptide. The formation of this backbone hydrogen bond brings Gln71, Ser184 and Glu187 of KIR2DL2 in such close proximity with the peptide that it constrains the size of the P8 side chain that would allow interaction with KIR to occur. To further examine the HLA-Cw3 peptide preference, a series of substitutions were introduced at the P8 position of GAV peptide. The altered peptides were refolded individually with HLA-Cw3 heavy chain and β2 microglobulin (β2m). The refolded class I molecules each bind to a class I MHC specific antibody w6/32 with similar affinity. Their binding to KIR2DL2, as measured by SPR, showed that the affinity decreases as the size of peptide at P8 increases. More specifically, amino acids larger than Val at the P8 position completely abolished the receptor binding (*Table 1*). In contrast, HLA-Cw3 complexed with the TAM peptide (see *Table 1*), different from GAV except at the P4, P8 and P9 positions, and the motif peptide AAA bind to the receptor with affinities similar to that of the wild-type GAV peptide. These results suggest that residues other than P7 and P8 do not contribute significantly to the receptor recognition, and secondly that Leu at P7 (GAV peptide) is only slightly more favored than an Ala or a Tyr at this position.

The 'upward projecting' conformation of the Leu side chain at the P7 position is a curious feature of the bound GAV peptide. A survey of the crystal structures of class I MHC complexed with nonamer peptides, both human and murine, indicates that the P7 position generally adopts a partially buried conformation with its side chain either orientated parallel to the floor of MHC peptide binding groove or buried within the floor. The percentage of side chain surface area buried at the P7 position varies from 67 to 100%. The P7 Leu of the GAV peptide in the KIR2DL2/HLA-Cw3 complex is, however, only 38% buried by HLA-Cw3. This is particularly significant considering the hydrophobic nature of Leu and the complete burial of the P7 Tyr observed in the structure of a homologous KIR ligand, HLA-Cw4 (*Fig.* 5) (72).

It is possible that KIR recognition induces a conformational change of the peptide such that the P7 position becomes more accessible to the receptor. Indeed, as a result of the

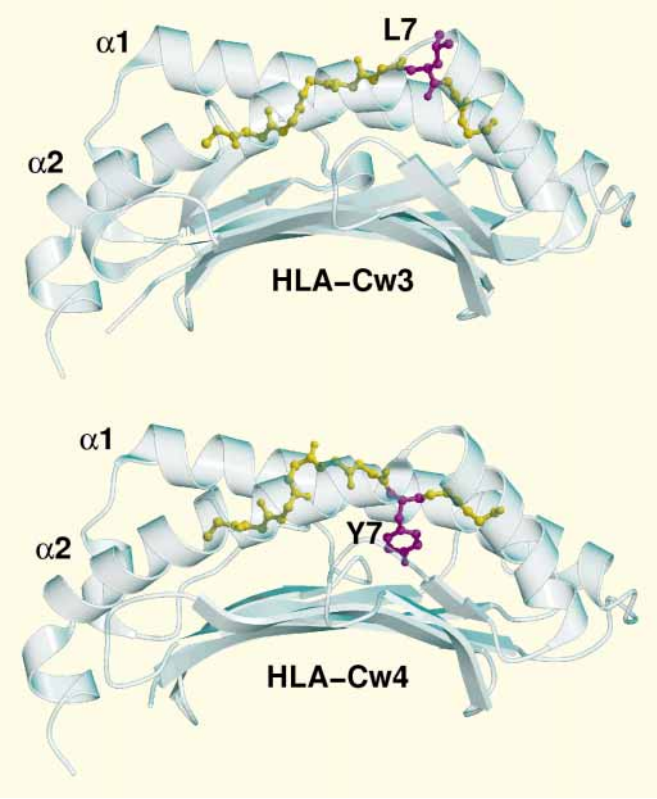

***Fig. 5.* Comparison of the HLA-Cw3 and -Cw4 $\alpha 1\alpha 2$ domains complexed with peptide.** The HLAs were made semi-transparent to allow viewing of the peptide backbones (yellow). HLA-Cw3 is complexed with the GAV peptide and HLA-Cw4 (PDB code 1QQD) is complexed with a nine residue consensus peptide. The side chains at the peptide P7 position of both structures are shown in magenta.

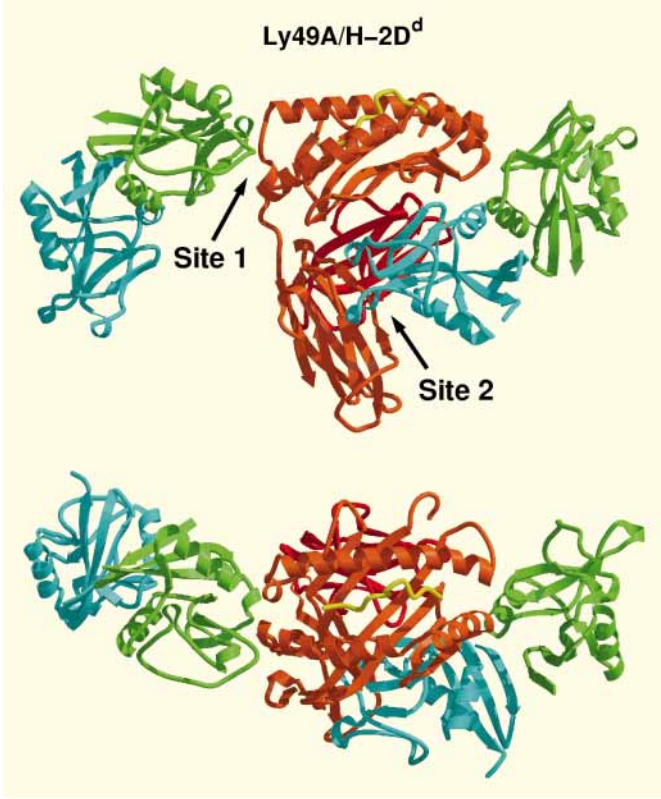

***Fig. 6.* The Ly49A/H-2D^d complex.** The top and bottom panels show two views rotated 90° about the horizontal axis. H-2D^d is brown and red with a yellow peptide and the two bound Ly49A homodimers are colored green and cyan (PDB code 1QO3).

hydrogen bonding between Gln 71 of KIR and the amide nitrogen of P8, the peptide backbone of GAV does adopt a slightly more puckered conformation than other peptides in the vicinity of P7 position (*Fig.* 5). A similar change in peptide conformation can also be found in a comparison of the structure of HLA-A2 complexed with the Tax peptide alone and the structure of HLA-A2 and the Tax peptide complexed with the A6 TCR (58).

Comparison between KIR and Ly49A in MHC recognition

Recently, the crystal structure of a complex between murine Ly49A and H-2D^d was determined (73). Unlike KIR2DL2, whose footprint on HLA-Cw3 superficially resembles that of TCR on the class I MHC molecules, Ly49A has two distinct binding sites on H-2D^d (*Fig.* 6). The first binding site is located at the N-terminal region of the class I MHC α1 helix and the second binding site is located below the H-2D^d peptide binding groove in a region that interfaces with α2, α3 and β2m of H-2D^d. Site 2 also partially overlaps with the binding site for CD8. No direct peptide contacts between Ly49A and H-2D^d are observed in either of the interfaces. Site 1 was favored initially, as the orientation of the receptor at this site is consistent with the positioning of the effector NK cells, even though site 2 buries a more extensive receptor–MHC interface. Recently, mutations in the regions of site 1 and 2 were produced to test the validity of each site. The results from Chung et al. (74) favored site 1 as the more likely binding site for Ly49A, whereas the opposite conclusion was reached by Matsumoto et al. (75) Consequently, further mutational and structural studies are needed in order to definitively resolve whether site 1, site 2 or both sites are important for the receptor recognition.

In any case, the molecular details underlying class I recognition by murine Ly49 receptors appear to be very different from those governing recognition by human KIR despite their functional similarities. It also remains to be seen whether the MHC binding mode of Ly49A is conserved among other Ly49

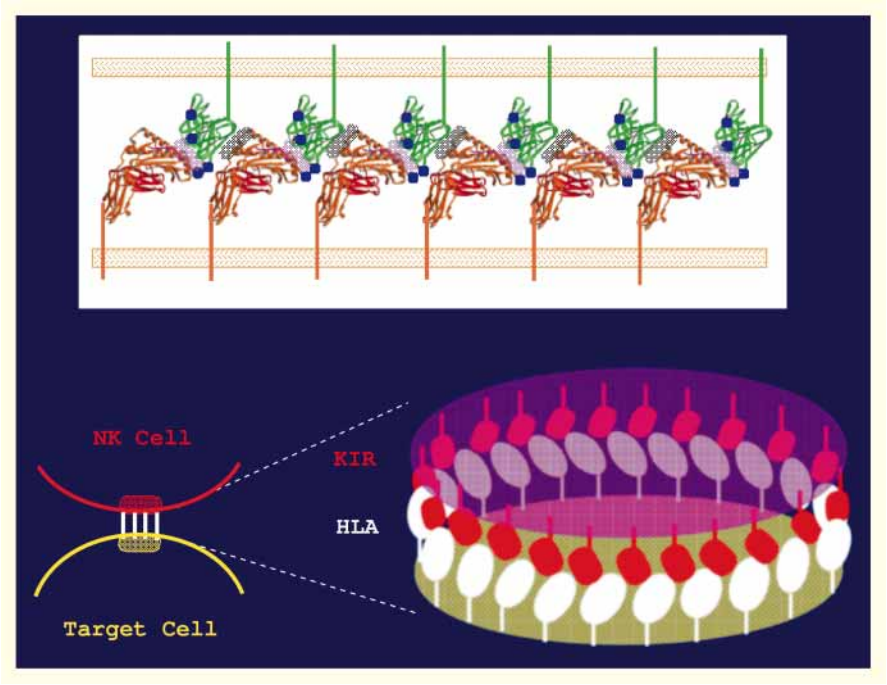

***Fig. 7.* A model for HLA-induced KIR clustering.** The top panel shows the oligomeric aggregate within the crystal lattice. HLA-Cw3 and KIR are brown and green respectively. The red and gray hashes highlight the functional and aggregate interfaces respectively. Predicted glycosylation sites are represented by blue dots at residues 63, 157 and 190 on KIR and residue 86 on HLA-Cw3. Horizontal brown hash above and below the aggregate depict the NK-cell and target cell membranes respectively. The bottom panel shows a cartoon depicting the KIR/HLA aggregate on the periphery of the NK-cell immune synapse.

molecules, such as Ly49C, which unlike Ly49A recognizes H2-K^b in a manner sensitive to the peptide sequence. Similarly, the question arises as to whether other class I-recognizing CTLRs, such as the CD94/NKG2 receptors, interact with class I in a manner that resembles that of Ly49A receptor with class I. In particular, all members of the CTLR superfamily of receptors appear to function as dimers, and the known structures of this superfamily share a conserved dimerization mode originally described in the structure of CD94 (73, 76, 77).

A model for KIR/HLA aggregation

Ligand-induced receptor oligomerization is presumed to be a common mechanism for initiating receptor-mediated signaling. The best understood examples to date are the growth hormone-mediated dimerization of human growth hormone receptor and the erythropoietin (EPO)-induced conformational change in the dimer of EPO receptor (78, 79). Since KIR receptors bear a structural resemblance to hematopoietic receptors, it has been proposed that KIR may form dimers upon binding to HLA in a manner similar to the growth hormone receptor. However, the crystal structure of KIR/HLA complex and the previous solution studies demonstrated a 1:1 stoichiometry for KIR/HLA interaction (49, 57). Recently, the development of digitized fluorescence imaging techniques has enabled the direct observation of both MHC-mediated TCR aggregation on T cells interacting with antigen-presenting cells (APC) and MHC-mediated KIR aggregation on NK cells interacting with target cells. The so-called immunological synapses at the interface of T cells and APC consist of a central cluster of TCR/MHC complexes and a peripheral ring of adhesion molecules (80, 81). In contrast, the observed NK-cell immune synapse is formed with a central leukocyte function-associated antigen-1/intercellular adhesion molecule-1 cluster and a peripheral KIR/HLA cluster in a shape of a donut (82). The question of how the KIR/MHC complex forms this ordered donut-shaped oligomeric aggregate remains unclear. One possible form of such an oligomer was reported in the crystal structure of KIR2DL2 (31). In that model, the receptors form a regular array through an interaction between the D1 domain of a receptor and the D2 domain of the preceding receptor. A second oligomerization form was observed in crystals of the KIR2DL2/HLA-Cw3 complex (57).

Within these crystals, each KIR molecule makes an additional contact, apart from the functional binding interface, with a symmetry-related HLA-Cw3 molecule in a peptide-independent manner. This KIR/HLA contact surface is formed between the B and E β-strands of the KIR D2 domain and the C-terminal end of the α2 helix of HLA-Cw3 (*Fig. 7*). The interface buries 530 $Å^2$ of surface area and is characterized by mostly van der Waals interactions. Interestingly, this KIR/HLA contact bridges adjacent complexes to form an oligomeric

KIR/HLA aggregate (*Fig. 7*). In this form of oligomer, the KIR/HLA complexes are all in the same orientation and the molar ratio between receptor and ligand is maintained at 1:1. Furthermore, the putative glycosylation sites on both KIR2DL2 and HLA-Cw3 are located away from the oligomerization interface. It is possible that this form of receptor–ligand oligomerization resembles the receptor clustering on the surface of NK cells. However, additional studies are needed to address the biological relevance of this oligomer, particularly its implications for receptor signaling.

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